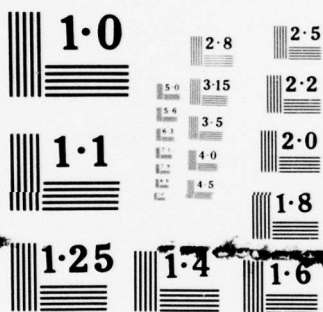


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TECHNICAL REPORT 7511

MUNITIONS-RELATED SUBSTANCES OF POTENTIAL CONCERN
AS AIRBORNE POLLUTANTS:
A PHYTOTOXICOLOGICAL EVALUATION

LYLE E. CRAKER, Ph.D.
JACK C. DACRE, Ph.D.

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DECEMBER 1975

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This report provides a summary review and evaluation of phytotoxicological literature of 11 compounds associated with U.S. Army Ammunition Plants. These compounds are: sulfur dioxide, ammonia, acetic acid and acetic anhydride, nitrous oxide, nitric oxide and nitrogen dioxide, methyl nitrate, nitromethane, tetranitromethane and the mononitrotoluenes. The estimated ambient concentrations for sulfur dioxide, acetic acid and nitrogen dioxide would not appear to pose a phytotoxic hazard. Because of lack of data, no conclusive statements can be made for the remaining compounds. Recommendations for further work on		

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20. Abstract (Continued)

these latter compounds have been made; a protocol for ascertaining the phytotoxicity of airborne environmental contaminants has also been described in an appendix to the report.

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INTRODUCTION

Airborne emissions from U.S. Army Ammunition manufacturing plants could pose a serious phytotoxic hazard. Presently identified compounds in these emissions include compounds previously shown injurious to vegetation as well as compounds for which effects on vegetation are unknown. An attempt has been made herein to evaluate the potential phytotoxic hazards that may be associated with munitions manufacturing operations. Based upon this evaluation, recommendations for future investigations are included that may be required to assist the Army Surgeon General in developing the data-base for air quality standards are included.

Substances evaluated were selected on the basis of their probable phytotoxic effects^{1,2} (SO_2 , NO , NO_2 and NH_3); their identification by the U.S. Army Materiel Command as potential major air pollutants^{3,4} (methyl nitrate, tetranitromethane, nitromethane, and mononitrotoluenes); or their release to the atmospheric environment in relatively large quantities⁵⁻⁷ (acetic acid, acetic anhydride, and N_2O). Federal secondary emission standards⁸ have been established for nitrogen dioxide (0.05 ppm - annual arithmetic mean) and sulfur dioxide (0.5 ppm - maximum 3 hour concentration, not to be exceeded more than once per year). New Mexico limits gaseous ammonia discharge to 25 ppm by volume in the gas stream.⁹ A review of federal laws and regulations, state laws and current developments disclosed no secondary standards for the remaining compounds considered.⁹⁻¹³

Each compound has been examined for its probable phytotoxic effects taking into consideration the chemical nature of the compound, atmospheric reactions that modify compound toxicity, compound concentrations and distribution, and vegetation within target areas.

A previous report in this series¹⁴ dealt with the toxicology of airborne munition wastes to humans and animals and has described their point sources, emission scatter and other characteristics. This report expands on previous ones by adding phytotoxicity implications.

Extensive literature searches were conducted on each compound to secure and evaluate any previous studies that would indicate compound phytotoxicity. Sources examined and key words are listed in Appendix A of this report. For compounds known to be phytotoxic and for which limiting regulations already exist, only brief summaries of the variables affecting phytotoxicity, injury signs and sensitive plant species are presented. The reader is referred to other literature reviews^{1,2,15} for a more complete description on the phytotoxicity of those compounds.

For injury to occur, compounds must make contact with plant tissue. This contact can be through vapors, aerosols, or through compounds dissolved in rain water or attached to particulate matter. Pollutants could be deposited on soil and absorbed through plant roots. Vegetation injury could occur near the source or some distance away depending upon pollutant concentration and dispersion. Mixtures of pollutants may be more phytotoxic than individual pollutants. While major emphasis was placed on phytotoxicity from gaseous contact of individual compounds with vegetation, reports indicating toxicity of compounds dissolved in water or attached to particulates have been included.

Accumulation of compounds in plant tissue could lead to toxicity in animals and humans through food and feed chains. Previous reports^{2,16,17} have indicated that some pollutants (SO_2 , NH_3 , N_2O , acetic acid) do become incorporated into plant tissue. These compounds have been used in normal plant nutrition, however, and are not considered toxic hazards. Accumulation of the remaining compounds considered is unknown and could be a factor in assessing the potential hazard of munition plant emissions.

SULFUR DIOXIDE

Sulfur dioxide (SO_2) is a known phytotoxic compound.^{1,18-21} National secondary standards for ambient air quality have been established for SO_2 at 0.5 ppm - maximum 3 hour concentration not to be exceeded more than once per year.⁸ The extent and level of SO_2 injury to plants is known to be influenced primarily by the SO_2 concentration, the duration of exposure, the presence of aerosols and ozone, and the sensitivity of the plant species.^{1,18-20} More work has been done on SO_2 effects on plants than probably any other pollutant. A recent comprehensive review is by Brandt and Heck (1968).¹⁵

Acute injury is indicated by dead leaf tissue between veins and along leaf margins.^{1,18,21} Chronic injury is generally indicated by brownish-red or bleached white areas on leaf blades.^{1,18,21} Undersurface silvery may appear as cells below the epidermis collapse from SO_2 damage. Injury can be caused by 0.3 ppm SO_2 for 8 hours and probably is due to a build-up of sulfite and sulfate ions inside the leaf from SO_2 interacting with intracellular water. Since SO_2 enters the plant leaf through the stomata, plants are most sensitive in the daylight when stomata are open.^{1,18} Plants have been found to be relatively more sensitive when leaves are low in sugar.¹⁸ Generally middle-aged leaves are more sensitive than older or younger leaves.

Oxidation of SO_2 in the atmosphere can lead to the formation of sulfuric acid aerosol. In contact with plant surfaces, the aerosol causes small punctate spots on leaves, generally on the upper leaf surface.¹ Ozone in the air acts synergistically with SO_2 to reduce the leaf injury threshold.^{1,18}

Some plants which are relatively sensitive to SO_2 are listed in Table 1.

AMMONIA

Ammonia is known to be phytotoxic to plants in relatively high concentrations.^{15,22,23} Field injury has been reported when anhydrous ammonia used as fertilizer contacts vegetation. There are no reports of acute injury symptoms due to slow release of ammonia from industrial plants.²³

Buckwheat, *Coleus*, sunflower, and tomato foliage are injured by 1 hour exposure to 40 ppm or 4 hour exposure to 16.6 ppm ammonia.²⁴ Signs of injury include tissue collapse and chlorophyll loss with leaves showing a cooked green appearance and becoming brown upon drying. Monocot leaves may develop bright red or purple pigmentation streaks in the leaves. Leaf and fruit tissue of some species may turn black under high concentrations (200-400 ppm NH_3).²²

Ammonia will inhibit nitrogen fixation,¹⁶ but probably this does not lead to plant destruction. Relative sensitivity of some species of plants to NH_3 are indicated in Table 2.

ACETIC ACID AND ACETIC ANHYDRIDE

Gaseous acetic acid has been demonstrated to be phytotoxic in some plants. Fumigation of red oak, common hawthorne, white dogwood and other woody plants with 0.0011 g/m³ of acetic acid reduced growth of the shoots.²⁵ Acetic acid vapor in high concentrations (1.0 to 1.5 g in 3 l at 0.5°C) may affect the dormancy and development of buds in *Hydrocharis* species.²⁶ Sugar cane growth was inhibited by volatilization of acetic acid from soil.²⁷

Acetic acid vapor has been reported to be absorbed and utilized by bean leaves during photosynthesis,¹⁷ but there was no indication of phytotoxicity.

TABLE 1. SELECTED PLANTS WHICH ARE RELATIVELY SENSITIVE
TO SULFUR DIOXIDE^a

CROPS		
Alfalfa <i>Medicago sativa</i> , L.	Cotton <i>Gossypium</i> , sp., L.	Soybean <i>Glycine max.</i> , Merr.
Barley <i>Hordeum vulgare</i> , L.	Oats <i>Avena sativa</i> , L.	Wheat <i>Triticum</i> , sp.
Bean, field <i>Phaseolus</i> , sp., L.	Rye <i>Secale cereale</i> L.	
Clover <i>Melilotus</i> & <i>Trifolium</i> , sp.	Safflower <i>Carthamus tinctorius</i> , L.	
GARDEN FLOWERS		
Aster <i>Aster bigelovii</i>	Four o'clock <i>Mirabilis jalapa</i> , L.	Verbena <i>Verbena canadensis</i> , Brit.
Bachelor's button <i>Centarea cyanus</i> , L.	Morning glory <i>Ipomoea purpurea</i> , Roth	Violet <i>Viola</i> , sp.
Cosmos <i>Cosmos bipinnatus</i> , Cau.	Sweet pea <i>Lathyrus odoratus</i> , L.	Zinnia <i>Zinnia elegans</i> , Lorenz
TREES		
Apple <i>Malus</i> , sp.	Larch <i>Larix</i> , sp.	Pine, Eastern white <i>Pinus strobus</i> , L.
Birch <i>Betula</i> , sp.	Mulberry <i>Morus microphylla</i> , Buckl.	Pine, ponderosa <i>Pinus ponderosa</i> , Laws
Catalpa <i>Catalpa speciosa</i> , Warder	Pear <i>Pyrus communis</i> , L.	Poplar, lombardy <i>Populus nigra</i> , L.
Elm, American <i>Ulmus americana</i> , L.		
GARDEN PLANTS		
Bean <i>Phaseolus vulgaris</i> , L.	Lettuce <i>Lactuca sativa</i> , L.	Spinach <i>Spinacea oleracea</i> , L.
Beet, table <i>Beta vulgaris</i> , L.	Okra <i>Hibiscus esculentus</i> , L.	Squash <i>Cucurbita maxima</i> , Duchesne

Table 1 continued

Broccoli <i>Brassica oleracea</i> var. <i>botrytis</i> , L.	Pepper (bell, chili) <i>Capsicum frutescens</i> L.	Sweet Potato <i>Ipomoea batatas</i> , Lam.
Brussel sprouts <i>Brassica oleracea</i> var. <i>gemmifera</i> , L.	Pumpkin <i>Cucurbita pepo</i> , L.	Swiss Chard <i>Beta vulgaris</i> var. <i>cicla</i> , L.
Carrot <i>Daucus carota</i> var. <i>sativa</i> , L.	Radish <i>Raphanus sativus</i> , L.	Turnip <i>Brassica rapa</i> , L.
Endive <i>Cichorium endivia</i> , L.	Rhubarb <i>Rheum rhaponticum</i> , L.	
WEEDS		
Bindweed <i>Convolvulus arvensis</i> , L.	Fleabane <i>Erigeron canadensis</i> , L.	Ragweed <i>Ambrosia</i> <i>artemisiifolia</i> , L.
Buckwheat <i>Fagopyrum sagittatum</i> , Gilib.	Lettuce, Prickly <i>Lactuca scariola</i> , L.	Sunflower <i>Helianthus</i> , sp.
Careless weed <i>Amaranthus palmeri</i> S. Wats.	Mallow <i>Malva parviflora</i>	Velvet-weed <i>Gaura parviflora</i> , Dougl.
Curly dock <i>Rumex crispus</i> , L.	Plantain <i>Plantago major</i> , L.	

a. Summary table from Barnett and Benedict (1970).¹⁸

TABLE 2. SENSITIVITY OF SELECTED PLANTS TO AMMONIA^a

SENSITIVE		
Mustard <i>Brassica juncea</i> , Coss.	Sunflower <i>Helianthus annuus</i> , L.	
INTERMEDIATE		
Buckwheat <i>Fagopyrum esculentum</i> , Moench.	Grass, annual blue <i>Poa annua</i> , L.	Tobacco <i>Nicotiana tabacum</i> , L.
Cheeseweed <i>Malva rotundifolia</i> , L.	Grass, Kentucky blue <i>Poa pratensis</i> , L.	Tomato <i>Lycopersicon esculentum</i> , Mill.
Coleus <i>Coleus</i> , sp.	Lambs-quarters <i>Chenopodium album</i> , L.	
RESISTANT		
Apple (fruit) <i>Malus</i> , sp.	Dandelion <i>Taraxacum officinale</i> , Weber	Peach (fruit) <i>Prunus persica</i> , Sieb. & Zucc.
Chickweed <i>Cerastium</i> , sp.	Nettle-leaf goosefoot <i>Chenopodium murale</i> , L.	Pigweed <i>Amaranthus retroflexus</i> , L.

a. Summary from Heck, et al. (1970).¹⁸

Aqueous solutions of acetic acid supplied to plants have been shown to be phytotoxic.²⁸⁻³⁰ Wheat root growth was reduced 80-86% by a 1.25 M solution of acetic acid.³¹ Addition of 5% acetic acid solution will reduce the vitamin C content of several vegetables and fruit.³²

Because of its rapid conversion to acetic acid in the presence of water,³³ acetic anhydride probably would be phytotoxic to the same extent as acetic acid.

NITROUS OXIDE

Gaseous nitrous oxide (N_2O) is reported to have no effects upon the seismic sensitivity of *Mimosa* plants³⁴ nor on numerous bacteria exposed to the gas.³⁵ Some bacteria and plants produce small amounts of nitrous oxide in normal metabolism.^{16,35} Nitrous oxide in high concentrations probably will inhibit nitrogen fixation in plants by acting as a competitive inhibitor for nitrogen.¹⁶

No available information implicates N_2O as a phytotoxic compound in the environment.

NITRIC OXIDE AND NITROGEN DIOXIDE

Nitric oxide (NO) and nitrogen dioxide (NO_2) are known phytotoxic compounds.³⁶⁻⁴¹ National secondary standards for ambient air quality have been established for NO_2 at 0.5 ppm.⁸ NO_2 appears to be more phytotoxic than NO.^{36,41} Concentration of the nitrogen oxide, duration of the exposure, light, and sensitivity of the plant species are reported to affect the extent of nitrogen oxide injury to vegetation.⁴¹

High concentrations of NO_2 (>25 ppm) coming in contact with vegetation will generally cause necrotic lesions on leaves and subsequent defoliation.^{1,36} Low levels of NO_2 (<1 ppm) over 10-22 days cause chlorosis of leaves and growth suppression.^{1,36} Phytotoxicity of NO_2 in the dark is almost twice the phytotoxicity of NO_2 in the light.¹ Differences in sensitivity among plant species are illustrated by tomato which is reported to be injured by 6 ppm NO_2 for 2 hours³⁶ and heath (*Erica carnea*) which is unaffected by exposure to NO_2 at 1000 ppm for 1 hour.⁴⁰

Necrotic lesions on plant leaves exposed to high concentrations appear as water-soaked areas on the upper surface. Tissue in these regions collapse and give the leaf small irregular necrotic patches, generally light brown or bronze in color. The lesions may occur in any area of the leaf but become most prominent at the apex and leaf margins. In addition, leaves may develop a waxy coating or glazed appearance. Severity of response on plant leaves varies with the age of leaves and can be more severe on old or young leaves depending upon plant species.³⁶ Defoliation occurs in citrus (*Citrus* sp.), azaleas and hibiscus (*Hibiscus* sp.).^{39,42}

Bean and tomato plants growing for 22 days in an atmosphere containing 0.5 ppm NO_2 showed a 25% reduction in dry weight as compared with controls not exposed to NO_2 .³⁷ Carbon dioxide absorption (necessary for photosynthesis) was depressed in tomato and bean plants by exposure to

NO at 4 to 10 ppm, although normal absorption returned following removal of NO from the environment.³⁶ Both NO and NO₂ inhibit photosynthesis at threshold concentrations of 0.6 ppm.⁴¹

Relative sensitivity of plants to nitrogen dioxide are indicated in Table 3.

Nitrogen oxides can contribute to formation of ozone (a known phytotoxic compound) by interacting with hydrocarbons in the air.⁴³

TABLE 3. SENSITIVITY OF SELECTED PLANTS TO NITROGEN DIOXIDE^a

SENSITIVE		
Azalea <i>Rhododendron</i> , sp.	Hibiscus <i>Hibiscus rosasinensis</i>	Sunflower <i>Helianthus annuus</i> , L.
Bean, pinto <i>Phaseolus vulgaris</i> , L.	Lettuce (head) <i>Lactuca sativa</i> , L.	Tobacco <i>Nicotiana glutinosa</i> , L.
Brittlewood <i>Melaleuca leucadendra</i>	Mustard <i>Brassica</i> , sp., L.	
INTERMEDIATE		
Cheeseweed <i>Malva parviflora</i> , L.	Dandelion <i>Taraxacum officinale</i> , Weber	Orange <i>Citrus sinensis</i> , Osbeck
Chickweed <i>Stellaria media</i> , Cyrill	Grass, annual blue <i>Poa annua</i> , L.	Rye <i>Secale cereale</i> , L.
RESISTANT		
Asparagus <i>Asparagus officinalis</i> , L.	Grass, Kentucky blue <i>Poa pratensis</i> , L.	Nettle-leaf goosefoot <i>Chenopodium</i> , sp.
Bean, bush <i>Phaseolus vulgaris</i> , L.	Heath <i>Erica</i> , sp.	Pigweed <i>Chenopodium</i> , sp.
Carissa <i>Carissa carandas</i>	Ixora <i>Ixora</i> , sp.	
Croton <i>Codiaeum</i> , sp.	Lambs-quarters <i>Chenopodium album</i> , L.	

a. As summarized by Taylor and MacLean (1970).³⁶

METHYL NITRATE

No information on phytotoxicity of gaseous methyl nitrate to higher plants was available. Reports^{44,45} indicate that methyl nitrate is mutagenic in *E. coli* bacteriophage T4B at concentrations less than 0.054 M. Methyl nitrate may undergo photolysis to produce NO_2 .⁴⁶

NITROMETHANE

Fumigation tests with nitromethane at 1 ppm produced no visible phytotoxic signs on spinach, endive, beets, oats or alfalfa.⁴⁷ No other information on the phytotoxicity of nitromethane was available. Nitromethane may undergo photolysis to produce NO_2 .⁴⁶

TETRANITROMETHANE

There is no available information on the phytotoxicity of gaseous tetranitromethane. Of primary concern may be the photolysis of tetranitromethane to give NO_2 ,⁴⁶ a known phytotoxic compound.

MONONITROTOLUENES

Limited information is available on the phytotoxicity of gaseous mononitrotoluenes. A report by Fant, *et al.*,⁴⁸ in 1923 indicated that gaseous mononitrotoluenes decreased the growth of germinating corn seedlings.

Other studies have reported the response of plants growing in aqueous solutions of mononitrotoluenes. Using duckweed, *Lemna perpusilla* Torr, Schott and Worthley (1974)⁴⁹ tested the phytotoxicity of *o*-nitrotoluene, 2,4-dinitrotoluene, 2,4,6-trinitrotoluene and 4-amino-2-nitrotoluene in concentrations of 0.01 to 100 ppm in comparison with phytotoxicity of 2,4-dichlorophenoxyacetic acid, a herbicide. A summary of the results are presented in Table 4.

In another study, *o*-, *m*-, and *p*-nitrotoluene are reported not to affect water microflora.⁵⁰ Application of *o*-nitrotoluene at 1000 liters per hectare has been used as an effective herbicide for irrigation canals.^{51,52}

TABLE 4. RESPONSE OF DUCKWEED TO TNT AND RELATED WASTES
IN AQUEOUS SOLUTION^a

Compound	Solution	Concentration of Compound (ppm)						
	pH	100	50	10	5	1	0.5	0.1 ^b
Response ^c								
o-Nitrotoluene	6.3	D	-	0	-	0	-	0
	8.5	0	0	0	-	0	-	-
2,4-Dinitrotoluene	6.3	D	-	D	-	X	X	0
	8.5	D	D	D	D	X	-	0
2,4,6-Trinitrotoluene	6.3	-	D	D	D	X	0	0
	8.5	-	D	D	D	X	-	0
4-Amino-2-nitrotoluene	6.3	D	X	0	-	0	-	-
	8.5	X	X	X	-	0	-	-

a. Data from Schott and Worthley (1974).⁴⁹

b. Concentration at which 2,4-dichlorophenoxyacetic is toxic.

c. D = death; X = decrease in growth rate; 0 = no effect; - = not tested.

DISCUSSION

Previous sections of this report have outlined the known and potential phytotoxicity of 11 compounds associated with U.S. Army munition manufacturing plants. A summary of the available phytotoxicity data is presented in Table 5. Data on ambient concentrations for nitrogen dioxide, sulfur dioxide, and acetic acid indicate that present levels probably are not phytotoxic (Table 6). However, increased plant operation undoubtedly would increase levels above the phytotoxic threshold. In addition, under certain atmospheric conditions the dilution of airborne pollutants that normally occurs between point sources and facility boundaries could be prevented thus causing infrequent but serious phytotoxic injury. Information is lacking for making any conclusive statement on the remaining compounds.

TABLE 5. SUMMARY OF AVAILABLE INFORMATION ON VISUAL PHYTOTOXIC SYMPTOMS OF POLLUTANTS

Pollutant	Symptoms	Type of Tissue Affected	Probable Injury Threshold		Reference
			Concentration	Duration	
Nitrogen dioxide (NO ₂)	Irregular, white or brown collapsed lesion on intercostal tissue and near leaf margin.	Middle-aged leaves	2.5 ppm	4 hr	1
Nitric oxide	Reduced CO ₂ uptake	Leaf	4 ppm	-	36
Sulfur dioxide (SO ₂)	Bleached spots, bleached areas between veins, bleached margin, chlorosis, growth suppression, early abscission, and reduction in yield.	Middle-aged leaves	0.3 ppm	8 hr	1
Ammonia (NH ₃)	Acute tissue collapse, cooked green appearance on leaves, necrotic spotting on leaf margins. Bleached or purple colored tissue.	Leaf	16.6 ppm	4 hr	36
Acetic acid	Reduced growth	Shoots	0.0011 g/m ³	-	25,27
Acetic anhydride	-	-	-	-	-
Mononitrotoluenes	Reduced growth	Germinating seed	Saturated	-	48
Methyl nitrite	Mutations	Bacteriophage	0.054 M	-	46
Nitromethane	-	-	1 ppm (est.)	-	47
Tetranitromethane	-	-	-	-	-

TABLE 6. ESTIMATED AMBIENT CONCENTRATIONS OF SELECTED
MILITARY-GENERATED AIR POLLUTANTS

Pollutant	Site	Estimated Concentration, mg/m ³	Location	Refer- ence
Acetic acid	HAAP, Area A	5.1	At boundary	14
	HAAP, Area B	1.3	At boundary	
Acetic anhydride	HAAP, Area A	0.58	At boundary	14
Methyl nitrate	HAAP, Area A	0.42	0.12 km outside boundary	14
	HAAP, Area B	0.27	At boundary	
Nitromethane	HAAP, Area A	0.026	0.12 km outside boundary	14
Tetranitromethane	VAAP	0.13	0.8 km outside boundary	14
	RAAP	0.48	At boundary	
Nitrous oxide	HAAP, Area B	3.0	At boundary	14
Mononitrotoluene	-	Unknown	-	-
Nitric oxide	VAAP	0.028 ppm (ave.)	Pond 5 outside boundary	54
Nitrogen dioxide	HAAP, Area A	0.090 ppm (max.)	At boundary	6
	HAAP, Area B	0.200 ppm (max.)	At boundary	
	VAAP	0.036 ppm (ave.)	Pond 5 outside boundary	
Sulfur dioxide	HAAP, Area A	0.255 ppm (max.)	At boundary	6
	HAAP, Area B	0.270 ppm (max.)	At boundary	
	VAAP	0.011 ppm (ave.)	Pond 5 outside boundary	
Ammonia	-	Unknown	-	-

RECOMMENDATIONS FOR FUTURE STUDY

Site inspections, by a qualified plant physiologist, should be conducted of the vegetation at appropriate locations in the spring and summer to locate any visual signs of phytotoxicity.

Initiation of test trials to determine the phytotoxicity of compounds (individually and collectively). Tests should include: acetic acid, acetic anhydride, methyl nitrate, tetranitromethane, nitromethane, and mononitrotoluenes.

Monitoring programs to ascertain the concentrations at selected production sites should be initiated for ammonia, nitrogen oxides, and the mononitrotoluenes.

Quantitative dispersion modeling for the prediction of ambient concentrations of air pollutants around production sites under various (normal and adverse) atmospheric conditions. Careful consideration must be given to downwash, fumigation, thermal inversion frequencies, and effects of surface topography. Thus, the work in reference 4 should be extended to cover conditions of importance in the prediction of phytotoxic injury.

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APPENDIX A

INFORMATION SOURCES CONSULTED FOR PHYTOTOXIC DATA

A. Reference Books

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B. Abstract Journals Searched

1. Chemical Abstracts, from 1907 through 1970 (Key Words: mononitrotoluenes, methyl nitrate, nitromethane, tetranitromethane, acetic acid, acetic anhydride, nitrous oxide, ammonia, explosives, munitions, phytotoxicity).
2. Biological Abstracts, from 1926 through 1970 (Key Words: mononitrotoluenes, methyl nitrate, nitromethane, tetranitromethane, acetic acid, acetic anhydride, nitrous oxide, ammonia, explosives, munitions, phytotoxicity).

C. Computer Searches

1. NTIS, 1964 to present (Key Words: mononitrotoluenes, methyl nitrate, nitromethane, tetranitromethane, acetic acid, acetic anhydride, nitrous oxide, ammonia, explosives, munitions, phytotoxicity, pink water, red water, dynamite, gunfire).
2. CAIN, 1970 to present (Key Words: mononitrotoluenes, methyl nitrate, nitromethane, tetranitromethane, acetic acid, acetic anhydride, nitrous oxide, ammonia, explosives, munitions, phytotoxicity, pink water, red water, dynamite, gunfire).
3. DDC, 1940 to present (Key Words: munition(s), explosive(s), dynamite, TNT, nitro compounds, tetranitromethane, mononitrotoluenes, nitrous oxide, nitrogen oxides, acetic acid, acetic anhydride, methyl nitrate, nitromethane, sulfur dioxide, pink water, red water, phytotoxicity, effects on plants, plant responses).

APPENDIX B

PROTOCOL FOR DETERMINING THE PHYTOTOXICITY OF GASEOUS ENVIRONMENTAL CONTAMINANTS

SUMMARY

A protocol for ascertaining the phytotoxicity of airborne environmental contaminants is described. The procedures outlined require a series of different studies, some prerequisite to others. Each portion of the protocol is designed to provide essential inputs for consistent ecological evaluation of pollutants, individually and if required, in combination.

The protocol is sectioned into tasks which progress from initial field evaluation to completed testing of pollutants for phytotoxicity. These tasks are labeled: (1) field studies and (2) greenhouse studies.

At the end of each task a decision point is reached and progression in or to a subsequent task is decided on the basis of previous results, program needs, and resource availability.

INTRODUCTION

Developing a general application protocol for identification of airborne phytotoxic contaminants necessarily limits the definitive features of these directives to suggested guidelines. Plant response to air pollutants depends not only upon specific characteristics of the plant and pollutants, but also upon a multitude of modifying factors within the environment *per se*. Innumerable combinations of plants and contaminants may be encountered under different environmental conditions.

Airborne pollutants can be described by their physical state, gaseous, aerosol or particulates. Pollutants in all forms can be injurious to plants, although the amount and type of vegetation damage may be different. Characteristics of gaseous pollutants may change as they become dissolved in absorptive moisture or attached to particulates. Mixtures of pollutants may interact to form new pollutants. [This protocol is designed for determining phytotoxicity of gaseous pollutants as gases.]

For vegetative damage to occur, the contaminant must be in contact with the plant. Some airborne pollutants are known to penetrate into plant tissue through stomates on aerial portions of plants and their phytotoxicity is limited to times when stomates are open. Other pollutants may pass directly through tissue surface layers. Contaminants dissolved in water or attached to particulates may contact vegetation as rain or dust settlements and initiate the phytotoxic response at the plant surface.

Higher plants are distinguished by their differences in form, habitat, and physiology. Wide variation in growth and development exist from one genus to another and significant differences may exist within cultivars of the same species. [Plant sensitivity to gaseous pollutants varies with plant types, plant environment and plant developmental stages.]

Certain background information must be available before this protocol can be employed effectively. [Not all contaminants in the air are necessarily harmful to plants.] Amounts, types, and effective release times of contaminants should be ascertained. Review of literature and other information sources on each contaminant should be utilized to identify the physical and chemical properties, the distribution and persistence in the environment and any known phytotoxic characteristics. Vegetation surveys should be used to identify primary and significant plant species within target areas.

TASK ONE: FIELD STUDIES

The objective of task one is to discern any indications of compound phytotoxicity on native flora. The best evidence of contaminant phytotoxicity is observation of definable plant injury at the target site. Results from this study will assist in selection of test plants for other tasks outlined in this protocol and will give indications of the extent of the pollution problem.

Experimental Procedures

Selected indigenous plant species of each area exposed to contaminants should be examined for expression of phytotoxic symptoms. Guidelines on field diagnosis of air pollution injury to plants are presented in other sources^{2,53} and are, therefore, not repeated in this protocol.

Observations must be completed during a plant's growing season so phytotoxic signs will easily be visualized. It would be preferable to make at least two visits to the contaminated area, one in spring after trees have leaves and a second visit approximately 4 to 6 weeks later. Observations should be made on plants in the immediate contaminant source area and primarily downwind from the source until a point where dispersion calculations indicate significant dilution of pollutants. In addition, upwind areas should be evaluated for signs of air pollution injury. The upwind area serves as a control planting not exposed to contaminants and should indicate if there have been plant population changes downwind from a suspected contaminant source or if other sources of contaminant exist within the area.

Color photographs should be made of all injury signs for use in comparison with phytotoxic signs produced in greenhouse trials. All affected plants should be identified.

Conclusions

Evidence of vegetation injury at source sites may indicate presence of phytotoxic compounds among chemicals released. Lack of vegetation damage does not preclude phytotoxicity of compound. Injury may be in the form of reduced yields, population modifications or other measurable changes.

TASK TWO: GREENHOUSE STUDIES

The objective of task two is to test the potential phytotoxicity of compounds through exposure of selected test plants to known concentrations of contaminants. Proof of phytotoxicity requires consistent demonstration of signs of injury under controlled conditions.

The type of initial test will depend upon the field evidence of phytotoxicity. If no phytotoxic signs are detected in the field, or in the absence of field observations, plants should be exposed to mixtures of suspected contaminant gases. (Certain interacting gases should not be present in the same mixture, e.g., ammonia and acetic acid.) If phytotoxic signs are detected in the field, plants should be exposed to individual contaminant gases.

Unfortunately, the cultural practices for growing each species of plant is specific, and no general method can be recommended successfully. Therefore, the USDA or State Agricultural Experiment Stations should be consulted concerning problems in specific plant culture. Growth of healthy plants under controlled conditions is not always simple and considerable attention to light, temperature, pest control, and other growth variables is required.

As a general rule, all dose-response data should be subjected to probit analysis. In addition, evaluation of combinations of contaminants for synergism or antagonism should be determined.

Selection of Plant Material

Plant material must be suitable for growth in greenhouses, sensitive to air pollutants and representative of plant species growing in locations under investigation. If possible, indigenous species which were determined to be injured by airborne contaminants or are particularly prominent within the area should be included. In addition, the selection of plant categories and specific species should utilize important economic plants of the area.

Test plants for compound phytotoxicity should include, but are not limited to, those listed in Table B-1. Selected plants should be grown in uniformly mixed soil under near optimum conditions of light, temperature and moisture for each species. However, it should be recognized that plants are sometimes more sensitive to air pollutants when growing under stress conditions.

Evidence of phytotoxicity is indicated upon comparison of plants exposed to contaminants (test plants) and to plants not exposed to contaminants (control plants).

TABLE B-1. SUGGESTED TEST PLANTS FOR USE IN
AIR POLLUTANT PHYTOXICITY STUDIES

Plant	Reason for Selection	Stage of Growth to Use
Beans, <i>Phaseolus vulgaris</i> , L.	Common air pollutant test plant, dicot, ease of yield trial	Seeding to harvest
Tobacco, <i>Nicotiana tabacum</i> , L.	Common air pollutant test plant, dicot	4 Leaf to 16 leaf
Eastern White Pine, <i>Pinus strobus</i> , L.	Common air pollutant test plant gymnosperm, woody species	Seedlings
Annual bluegrass, <i>Poa annua</i> , L.	Common air pollutant test plant, monocot	Seed to maturity

Conclusions

Evidence of vegetation injury (visual or other) indicates (1) one of the individual components in the mixture to be phytotoxic; (2) two or more components are additive or synergistic; or (3) chemical transformation in the mix has produced one or more new compounds that are phytotoxic. Further tests outlined in Phase II of this task should be conducted. The absence of vegetation injury (visual or other) would seem to preclude any phytotoxicity among the compounds tested and no further tests would be necessary.

Experimental

Phase I - Mixture of Compounds. (To be used where there is no evidence of phytotoxicity in the field.)

Matched and paired greenhouses should be selected for growth and exposure of plants to contaminants. One house of each pair to be designated a control greenhouse and the other a test greenhouse. The control house(s) to be free of any gaseous pollutants and the test house(s) to contain only gaseous pollutants under study. Pollutants should be added to the air of the test house(s) so that the contaminants are equally dispersed throughout the plant growing area. Monitoring and control systems should maintain a fixed level of all pollutants in the test greenhouses. Throughout the test trials contaminant levels should be at a minimum of twice the monitored level of pollutants in the field. (Plants may be more or less sensitive under ambient conditions.)

Matched sets of plants should be placed in each of the greenhouses and grown through the designated stages (Table B-1). All sets of test plants should be replicated sufficiently to allow statistical analyses of phytotoxicity data. Plants should be observed regularly in detail for visual signs of toxicity. All visual signs should be recorded using color photographs and other available data collection techniques. Information on seed set and yields should be collected at maturity and direct comparison of test and control plants made to indicate detrimental effects of contaminants on those parameters.

Phase II - Single Compounds. (To be used if there is evidence of compound phytotoxicity in the field studies or Phase I of greenhouse studies.)

Growth of plants, greenhouses, and pollutant test conditions should be as outlined in Phase I, except individual contaminants should be utilized in the test greenhouse(s). Initial tests in Phase II should utilize contaminant concentrations twice the monitored levels in the field. Any contaminants that produce phytotoxic signs (visual or other) should be tested further under conditions previously outlined

in this phase but with the use of multiple contaminant levels in order to obtain quantitative data for establishing reliable dose-response curves. Data collection is to be as previously described in Phase I.

Conclusions

Evidence of vegetation injury (visual or other) indicates compound phytotoxicity. Threshold values are established by use of multiple compound concentrations. Absence of vegetation injury (visual or other) indicates that when tested individually the compound exhibits no phytotoxicity.

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